Figure 1

SEQ ID NO: 1

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 241 gatttatgga gaaagatgca gtcagaggaa atgatacgtt tgtaacactc gatgaaattt
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 361 aaaataagcc ctcaaggaaa acattacata cctgcctcga gttattaaga aaatattgta
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2101 ttgaggtaga tgaatcagat gtggaagaag acatttttcc taccacttca aagacagatc
2161 aaaggtggtc cagcacatca tccagcaaaa tcatgtccca gagtcaagta tcgaaagggg
2221 ttgattttga atcaagtgag gatgatgatg atgatccttt tatgaacact agttctttaa
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2341 gaaaatgtta caagctaaga gtttacagtt taagatttta agtattgttt cctgagcata
2401 actccataag taagaaattt ctagttcaca gacatacaat agcattgatt caccttgttt
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2521 aataaagcta ccatttt
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Figure 2

SEQ ID NO:2

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121 pvfsihgnhd dptgadalca ldilscagfv nhfgrsmsve kidispvllq kgstkialyg
181 lgsipderly rmfvnkkvtm lrpkedensw fnlfvihqnr skhgstnfip eqflddfidl
241 viwgheheck iaptkneqql fyisqpgssv vtslspgeav kkhvgllrik grkmnmhkip
301 lhtvrqffme divlanhpdi fnpdnpkvtq aiqsfcleki eemlenaere rlgnshqpek
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541 saddlmsidl aeqmandsdd sisaatnkgr grgrgrrggr gqnsasrggs qrgradtgle
601 tstrsrnskt avsasrnmsi idafkstrqq psrnvttkny sevievdesd veedifptts
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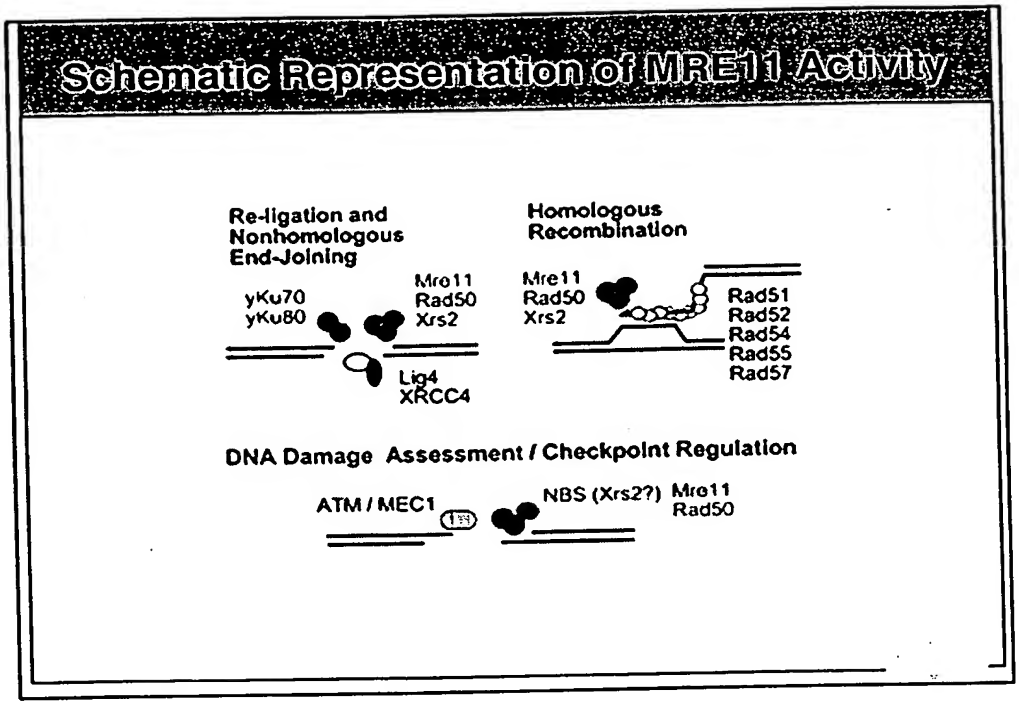
Figure 3



10025331 .122401

Figure 4

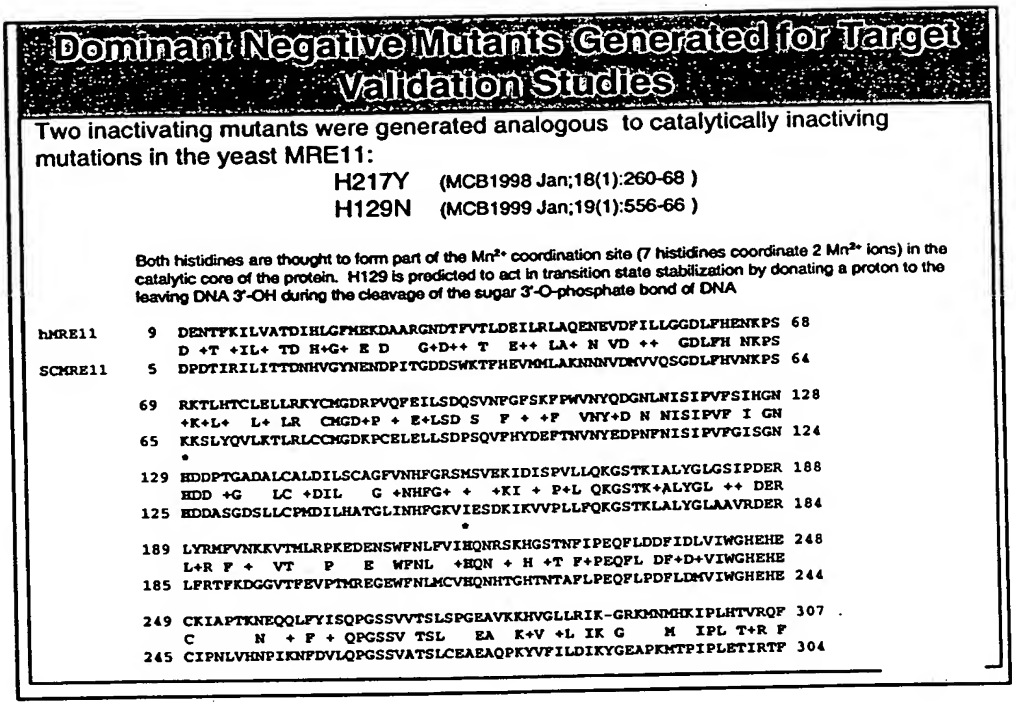
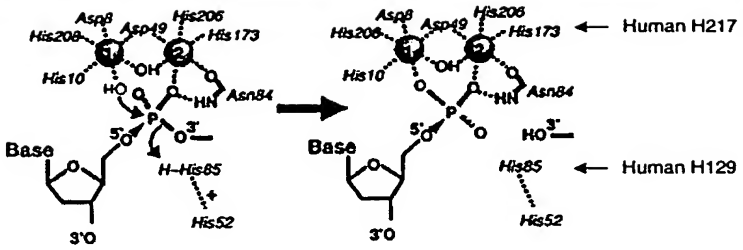

Dominant Negative Mutants Generated for Target Validation Studies
Two inactivating mutants were generated analogous to catalytically inactiving mutations in the yeast MRE11:
H217Y (MCB1998 Jan;18(1):260-68)
H129N (MCB1999 Jan;19(1):556-66)
Both histidines are thought to form part of the Mn2+ coordination site (7 histidines coordinate 2 Mn2+ ions) in the catalytic core of the protein. H129 is predicted to act in transition state stabilization by donating a proton to the leaving DNA 3'-OH during the cleavage of the sugar 3'-O-phosphate bond of DNA
hMRE11 9 DENTFKILVATDIHLGFMEKDAARGNDTFVTLDEILRLAQENEVDFILLGGDLFHENKPS 68
D +T +IL+ TD H+G+ E D G+D++ T E++ LA+ N VD ++ GDLFH NKPS
SCMRE11 5 DPDTIRILITTDNHVGYNENDPITGDDSWKTFHEVMMLAKNNNVDMVVQSGDLFHVNKPS 64
69 RKTLHTCLELLRKYCMGDRPVQFEILSDQSVNFGFSKFPWVNYQDGNLNISIPVFSIHGN 128
+K+L+ L+ LR CMGD+P + E+LSD S F + +F VNY+D N NISIPVF I GN
65 KKSLYQVLKTLRLCCMGDKPCELELLSDPSQVFHYDEFTNVNYEDPNFNISIPVFGISGN 124
•
129 HDDPTGADALCALDILSCAGFVNHFGRSMSVEKIDISPVLLQKGSTKIALYGLGSIPDER 188
HDD +G LC +DIL G +NHFG+ + +KI + P+L QKGSTK+ALYGL ++ DER
125 HDDASGDSLLCPMDILHATGLINHFGKVIESDKIKVVPLLFQKGSTKLALYGLAAVRDER 184
•
189 LYRMFVNKKVTMLRPKEDENSWFNLFVIHQNRSKHGSTNFIPEQFLDDFIDLVIWGHEHE 248
L+R F + VT P E WFNL +HQN + H +T F+PEQFL DF+D+VIWGHEHE
185 LFRTFKDGGVTFEVPTMREGEWFNLMCVHQNHTGHTNTAFLPEQFLPDFLDMVIWGHEHE 244
249 CKIAPTKNEQQLFYISQPGSSVVTSLSPGEAVKKHVGLLRIK-GRKMNMHKIPLHTVRQF 307
C N + F + QPGSSV TSL EA K+V +L IK G M IPL T+R F
245 CIPNLVHNPIKNFDVLQPGSSVATSLCEAEAQPKYVFILDIKYGEAPKMTPIPLETIRTF 304

Figure 5

Representation of Active Site of *P. furiosus* MRE11



Mn^{2+} coordination site 7 histidines coordinate 2 Mn^{2+} ions in the catalytic core of the protein.

H129 is predicted to act in transition state stabilization by donating a proton to the leaving DNA 3'-OH during the cleavage of the sugar 3'-O-phosphate bond of DNA

Mutations Generated for Dominant Negative Studies correspond to:

P. furiosus	***Human***
H173	H217
H85	H129

Figure 6

Summary of Target Validation Studies: MRE11

Dominant negative studies

	Antiproliferative Activity						
	Tumor				Normal		
	A549	Hela	PC3	H1299	HMEC	HUVEC	PrEC
Wt							
GFP-fusion	-	-	-	-	-	-	-
IRES GFP	-	-	nd	nd	-	-	nd
H217Y							
GFP-fusion	-	-	-	-	-	-	-
IRES GFP	-	-	nd	nd	-	-	nd
H129N							
GFP-fusion	++	++	-/+	-/+	-	-	-
IRES GFP	+	-	nd	nd	-	-	nd

Antisense: **A549 inconclusive**

(+ indicates antiproliferative effect in either the GFP positivity study, cell tracker or antisense studies)

Figure 7

Summary of Target Validation Studies: MRE11

Dominant negative studies

	Chemosensitization Activity		
	Tumor A549	**Hela**	**HMEC**
Wt GFP-fusion	-	-	-
H217Y GFP-fusion	++	++	-

Figure 8

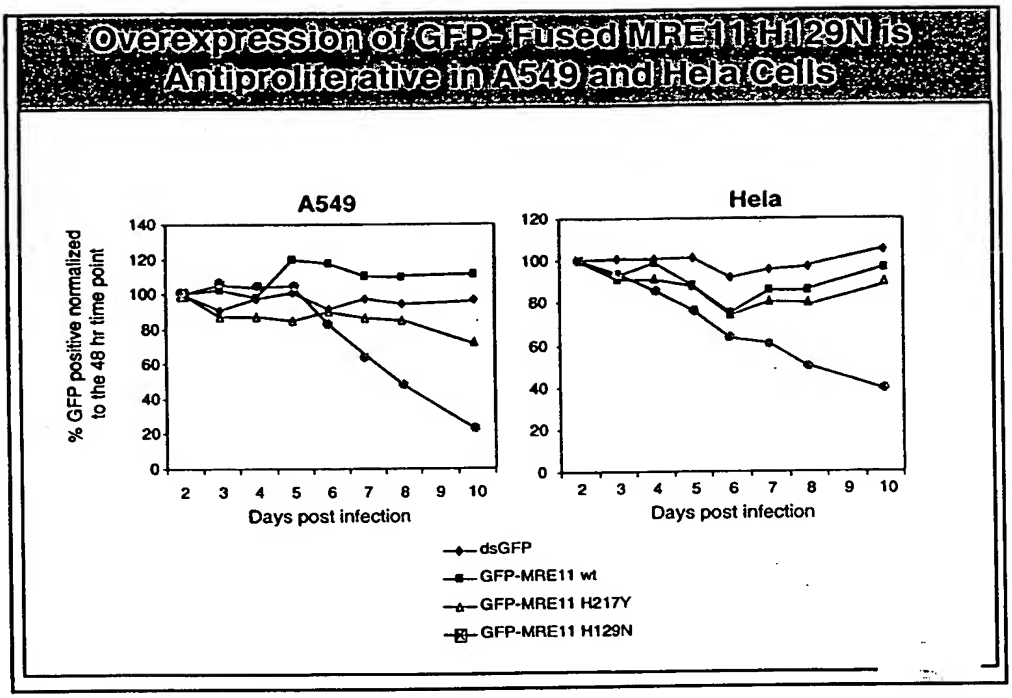

Overexpression of GFP-Fused MRE11 H129N is Antiproliferative in A549 and Hela Cells
A549
Hela
% GFP positive normalized to the 48 hr time point
Days post infection
Days post infection
dsGFP
GFP-MRE11 wt
GFP-MRE11 H217Y
GFP-MRE11 H129N

Figure 9

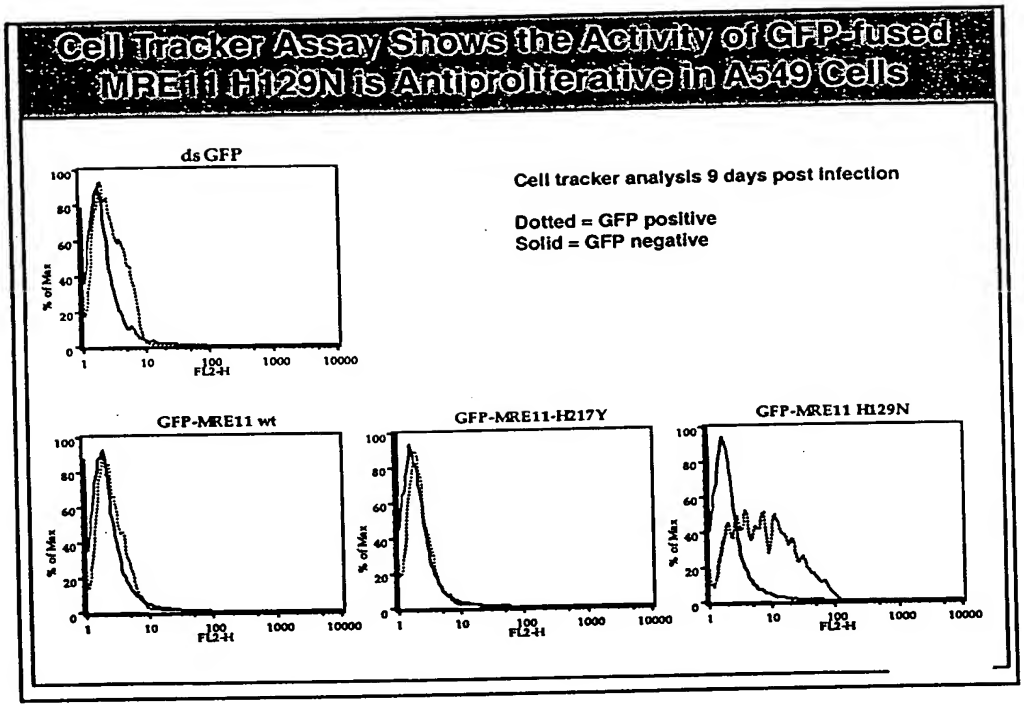

Cell Tracker Assay Shows the Activity of GFP-fused MRE11 H129N is Antiproliferative in A549 Cells
ds GFP
Cell tracker analysis 9 days post infection
Dotted = GFP positive
Solid = GFP negative
GFP-MRE11 wt
GFP-MRE11-H217Y
GFP-MRE11 H129N
% of Max
FL2-H
1
10
100
1000
10000
0
20
40
60
80
100

Figure 10

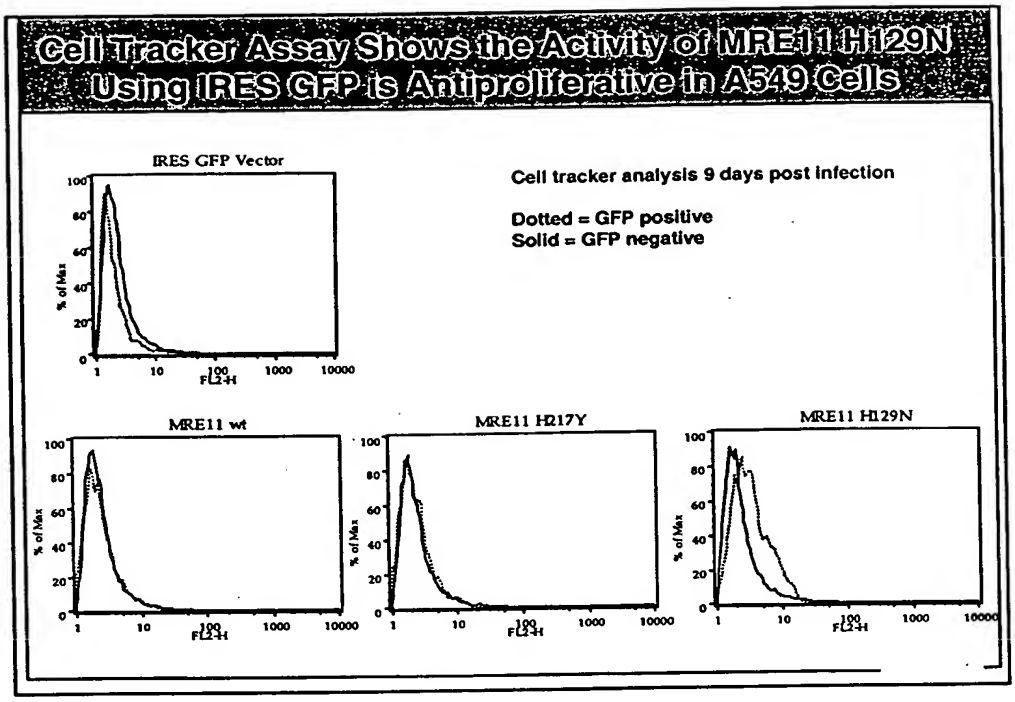

Cell Tracker Assay Shows the Activity of MRE11 H129N Using IRES GFP is Antiproliferative in A549 Cells
IRES GFP Vector
Cell tracker analysis 9 days post infection
Dotted = GFP positive
Solid = GFP negative
MRE11 wt
MRE11 H217Y
MRE11 H129N
% of Max
FL2-H
1
10
100
1000
10000
0
20
40
60
80
100

Figure 11

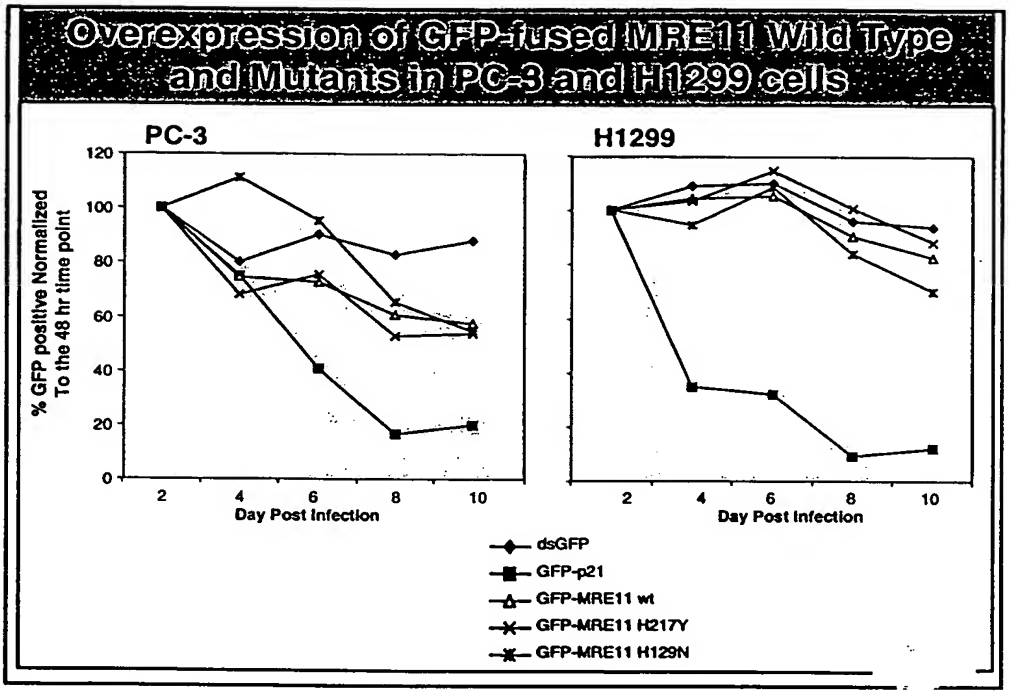

Overexpression of GFP-fused MRE11 Wild Type and Mutants in PC-3 and H1299 cells
PC-3
H1299
% GFP positive Normalized To the 48 hr time point
120
100
80
60
40
20
0
2
4
6
8
10
Day Post Infection
dsGFP
GFP-p21
GFP-MRE11 wt
GFP-MRE11 H217Y
GFP-MRE11 H129N

Figure 12

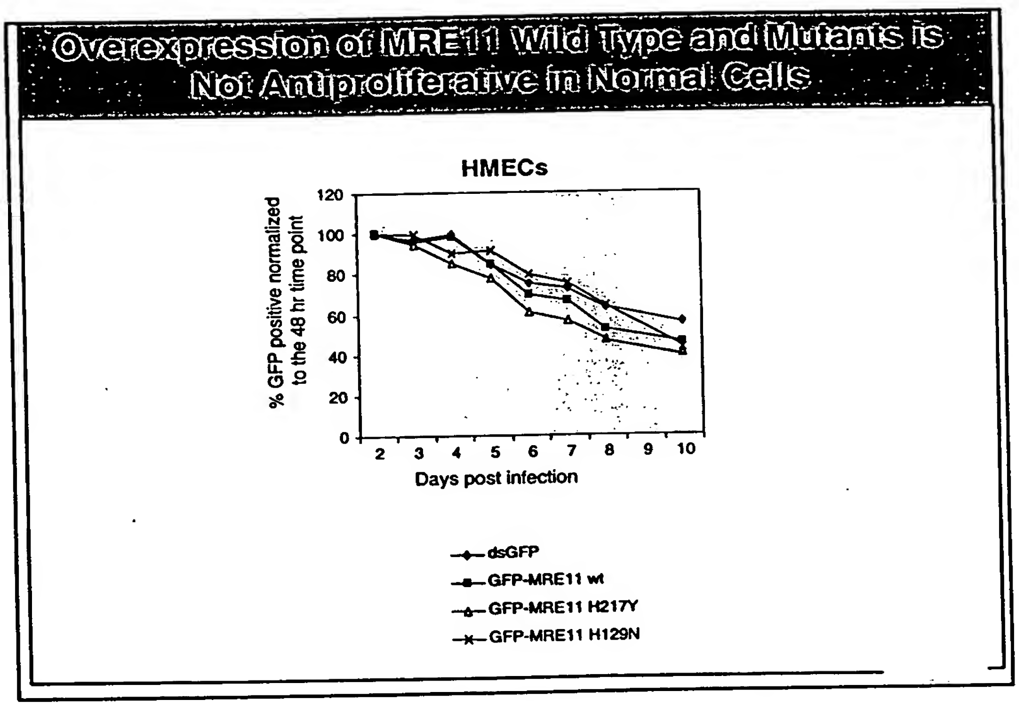


Figure 13

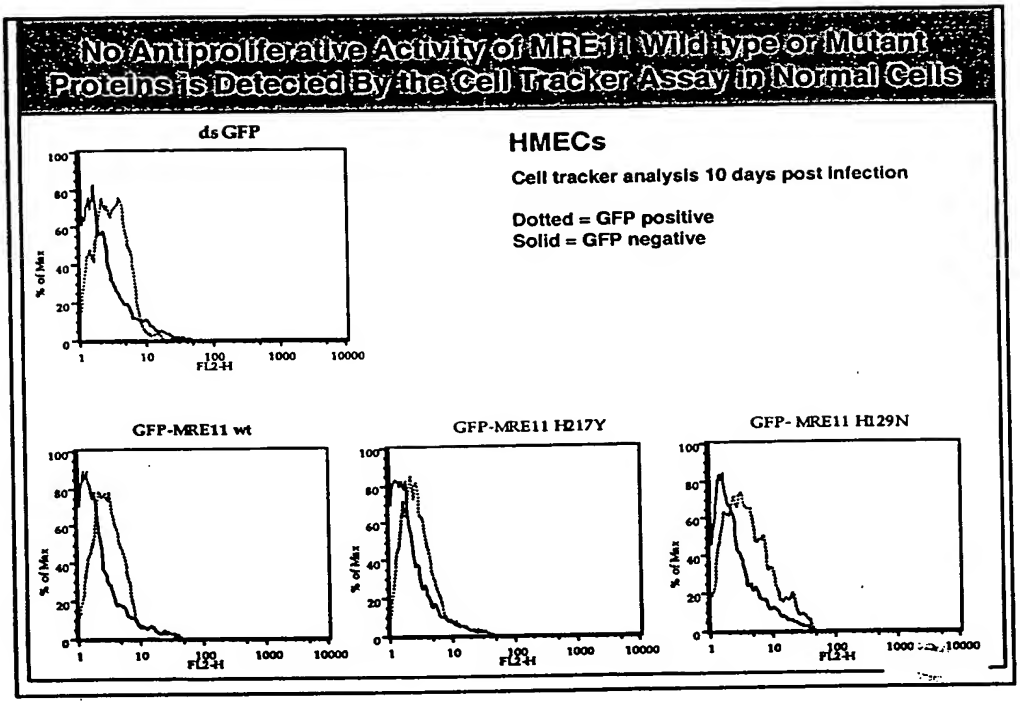

No Antiproliferative Activity of MRE11 Wild type or Mutant Proteins is Detected By the Cell Tracker Assay in Normal Cells
ds GFP
HMECs
Cell tracker analysis 10 days post infection
Dotted = GFP positive
Solid = GFP negative
GFP-MRE11 wt
GFP-MRE11 H217Y
GFP- MRE11 H129N
% of Max
FL2-H

Figure 14

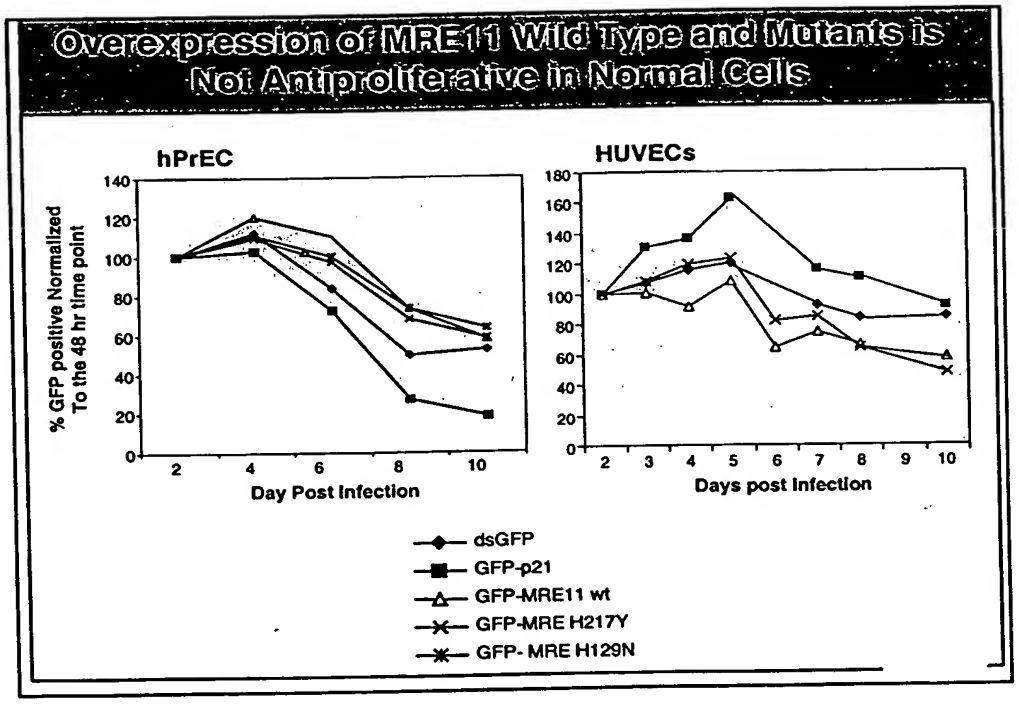

Overexpression of MRE11 Wild Type and Mutants is Not Antiproliferative in Normal Cells
hPrEC
HUVECs
% GFP positive Normalized To the 48 hr time point
Day Post Infection
Days post Infection
dsGFP
GFP-p21
GFP-MRE11 wt
GFP-MRE H217Y
GFP- MRE H129N

Figure 15

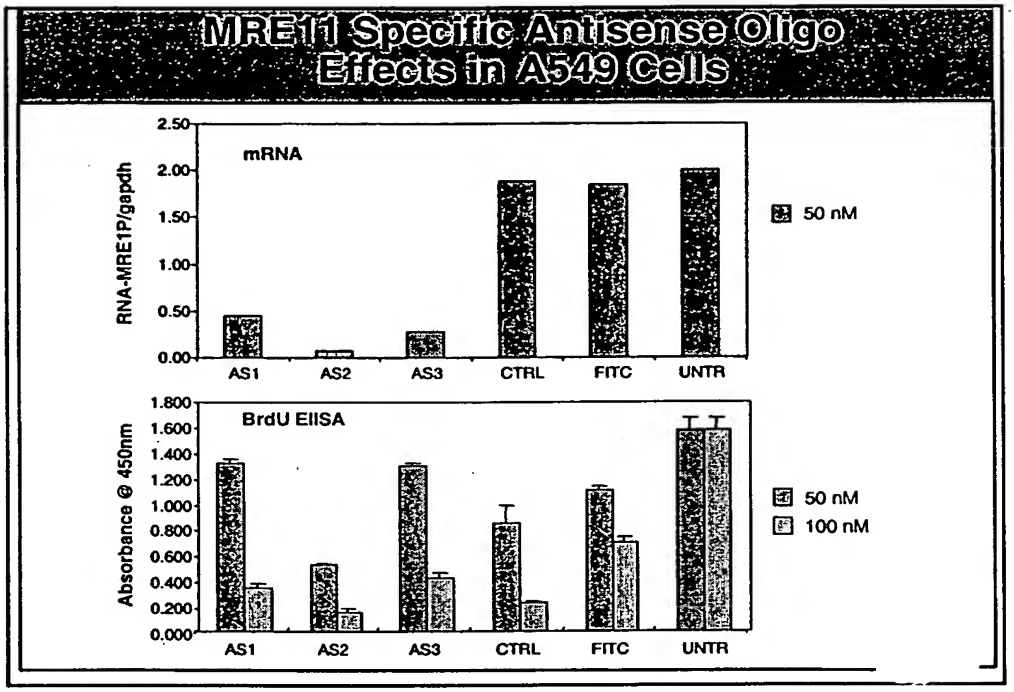


Figure 16

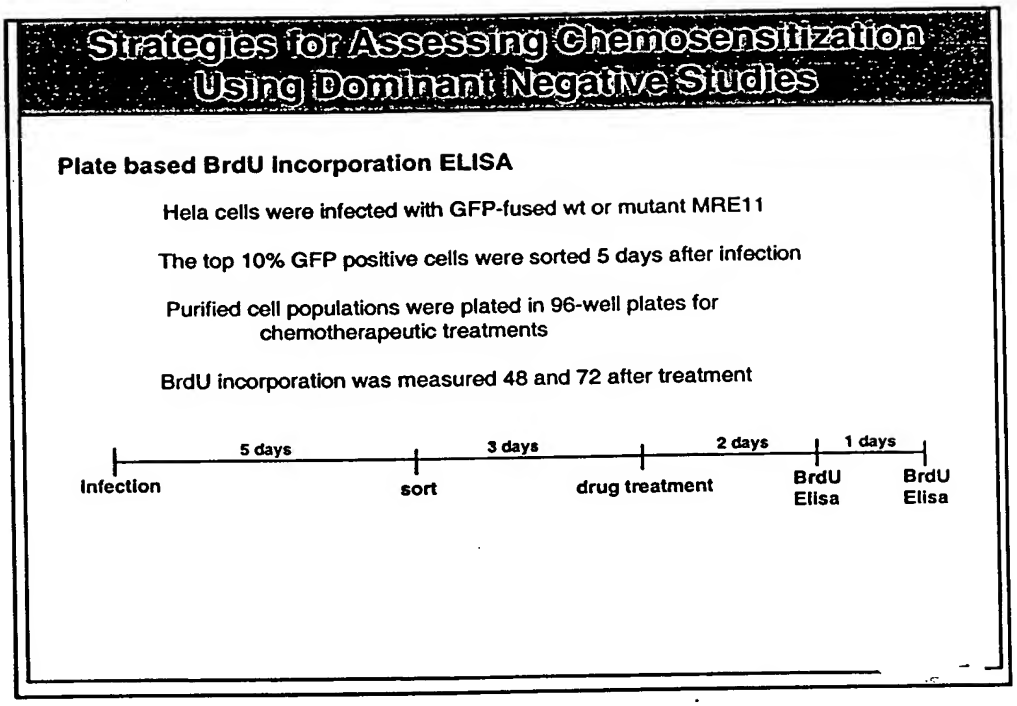
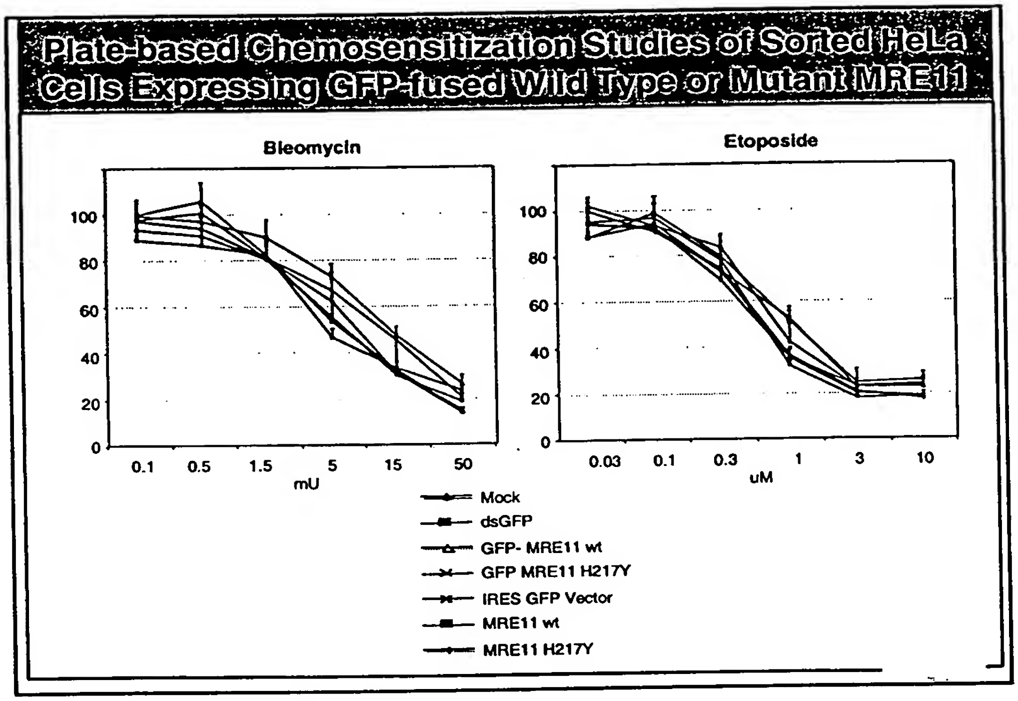


Figure 17



10025331.122101

Figure 18

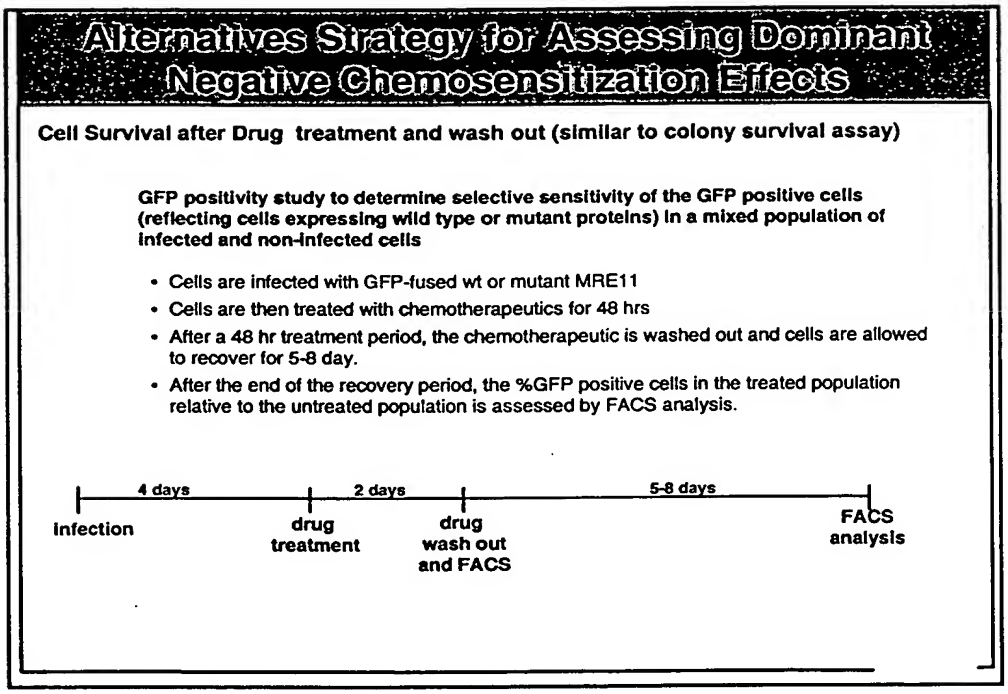

Alternatives Strategy for Assessing Dominant Negative Chemosensitization Effects
Cell Survival after Drug treatment and wash out (similar to colony survival assay)
GFP positivity study to determine selective sensitivity of the GFP positive cells (reflecting cells expressing wild type or mutant proteins) in a mixed population of infected and non-infected cells
• Cells are infected with GFP-fused wt or mutant MRE11
• Cells are then treated with chemotherapeutics for 48 hrs
• After a 48 hr treatment period, the chemotherapeutic is washed out and cells are allowed to recover for 5-8 day.
• After the end of the recovery period, the %GFP positive cells in the treated population relative to the untreated population is assessed by FACS analysis.
4 days
2 days
5-8 days
Infection
drug treatment
drug wash out and FACS
FACS analysis

Figure 19

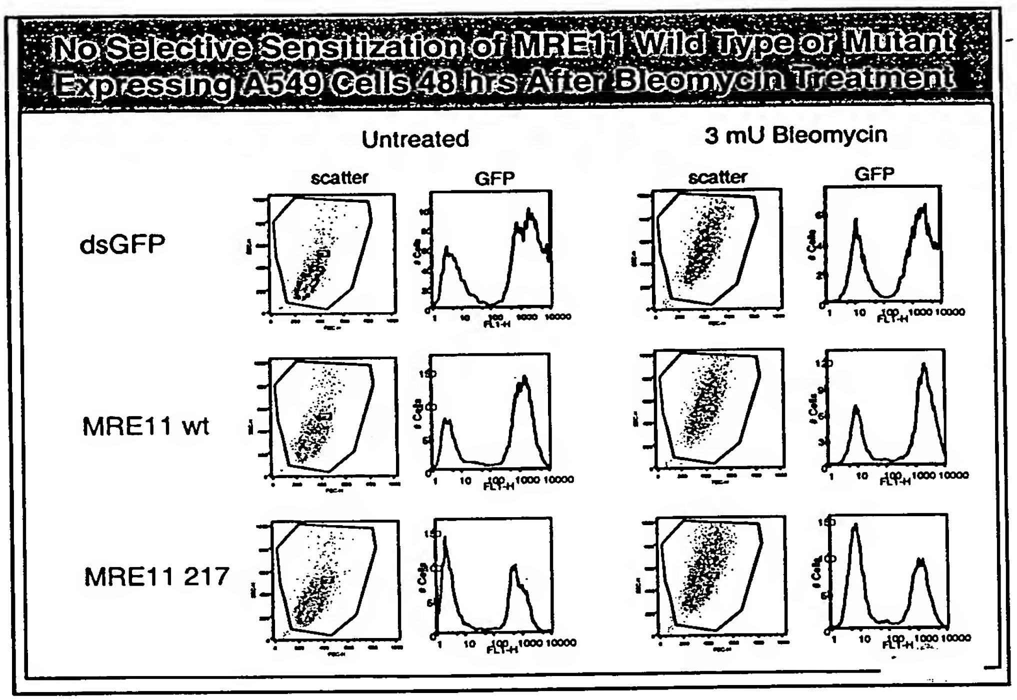


Figure 20

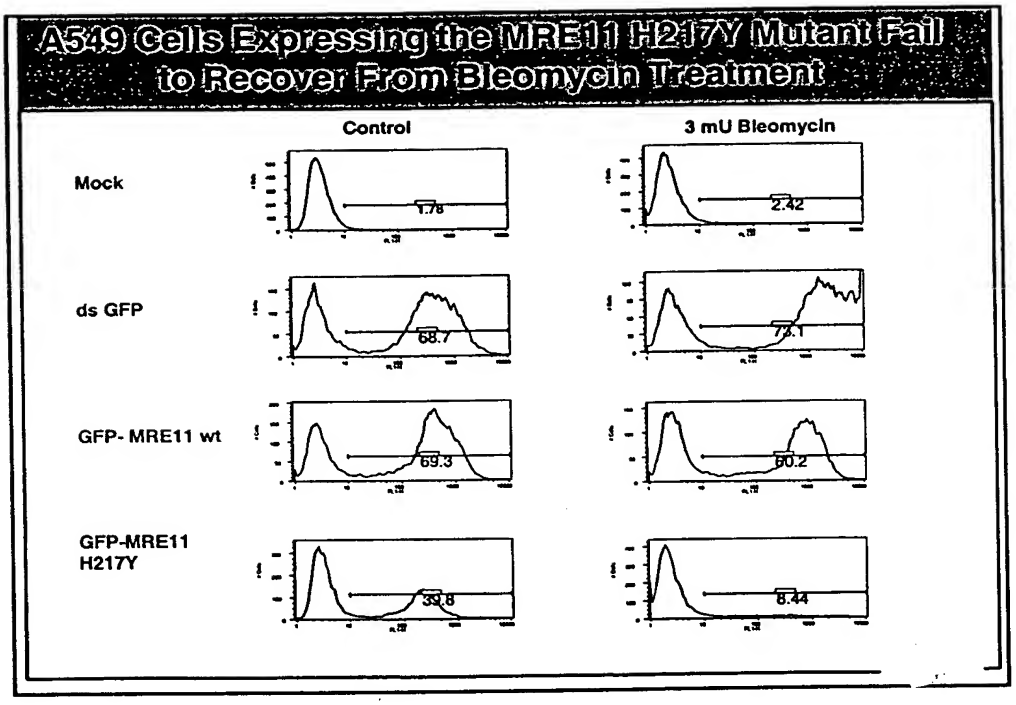


Figure 21

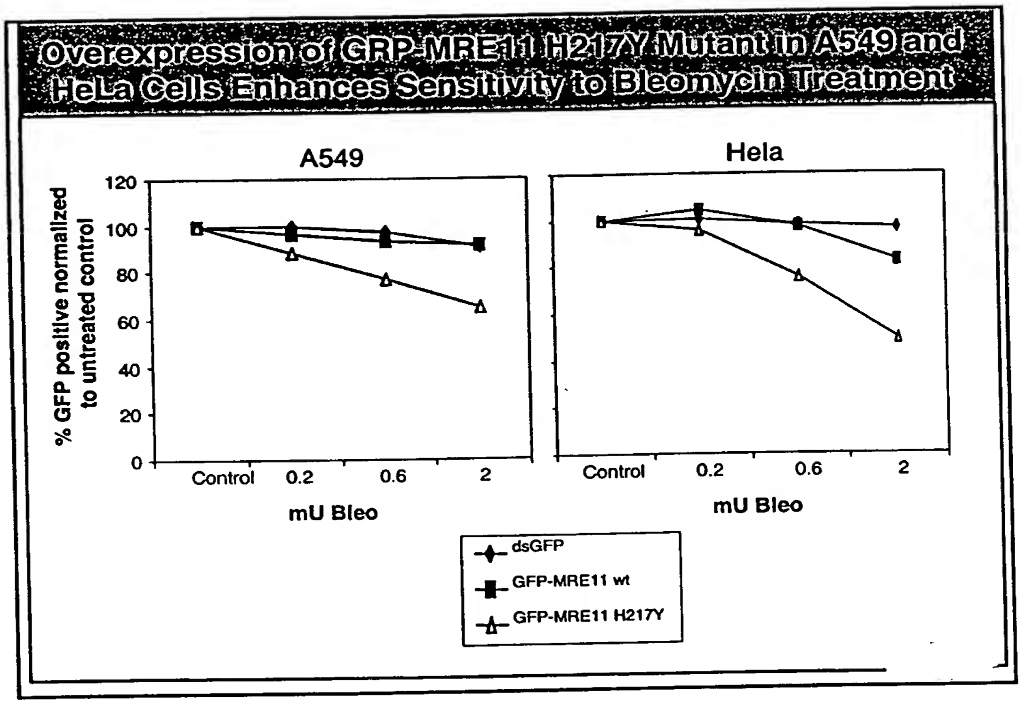


Figure 22

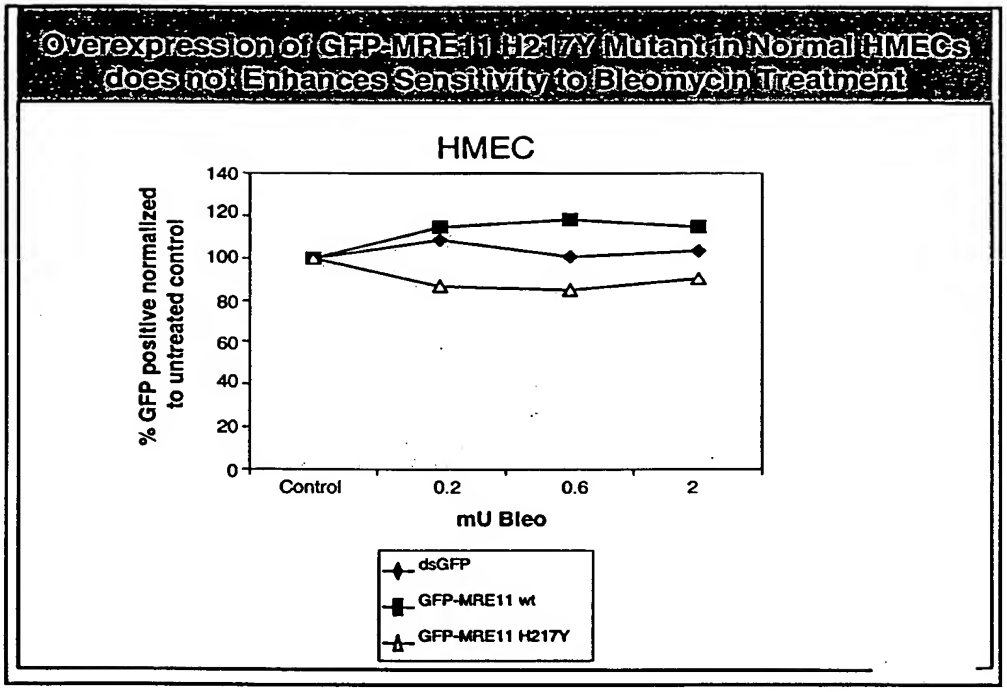
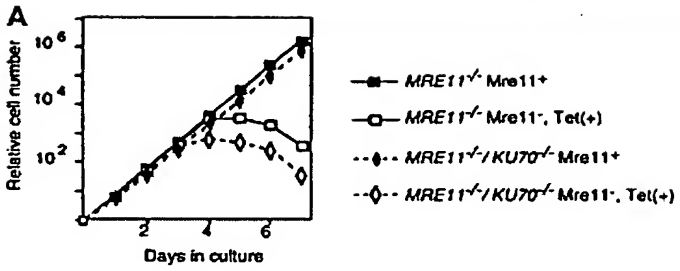

Overexpression of GFP-MRE11 H217Y Mutant in Normal HMECs does not Enhances Sensitivity to Bleomycin Treatment
HMEC
% GFP positive normalized to untreated control
140
120
100
80
60
40
20
0
Control
0.2
0.6
2
mU Bleo
dsGFP
GFP-MRE11 wt
GFP-MRE11 H217Y

Figure 23

Depletion of MRE11 is Antiproliferative in the Hyper-recombinogenic Chicken B-cell line DT40 Made Conditionally Null for MRE11



Additional phenotypes observed in MRE -/- cells

- chromosomal aberrations
- centrosome amplification
- enhanced sensitization to ionizing radiation

EMBO J. 1999 Dec 1;18(23):6619-29.

Figure 24

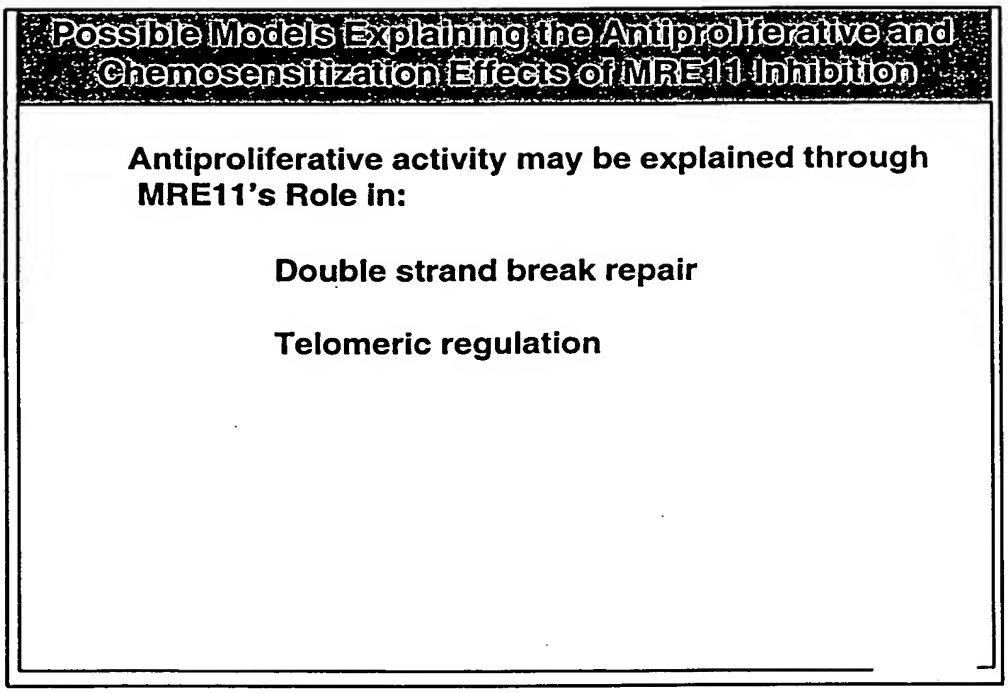


Figure 25

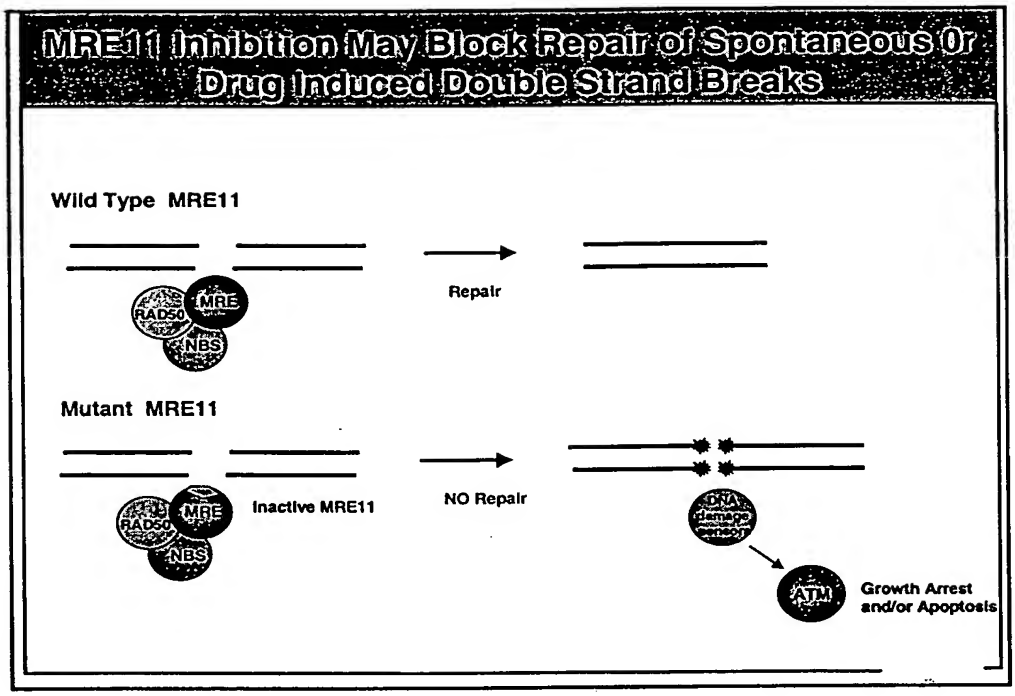
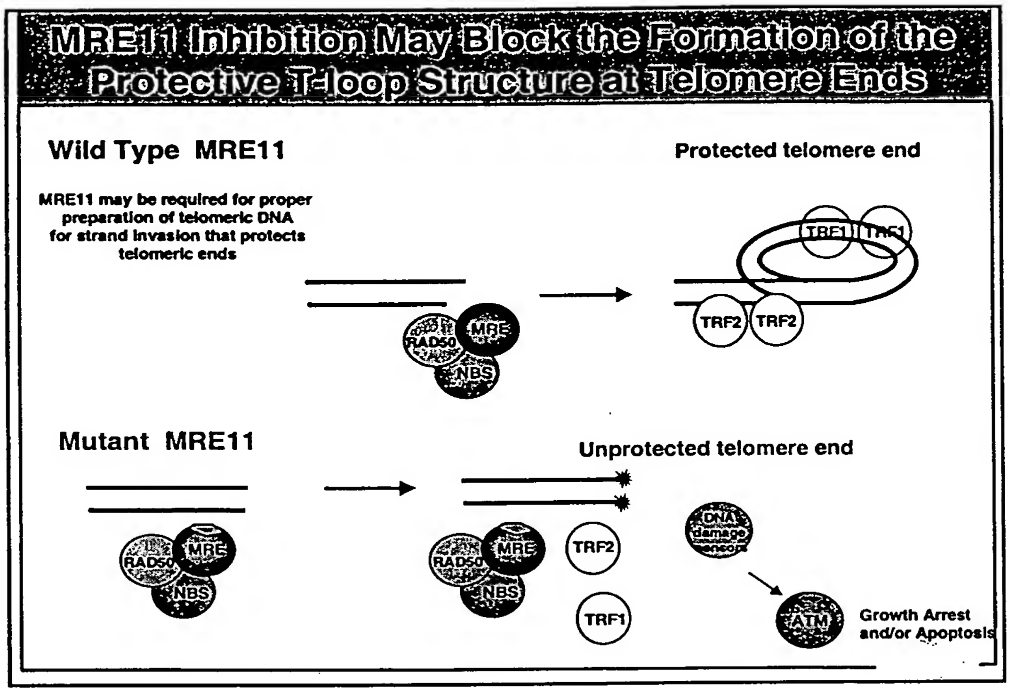

MRE11 Inhibition May Block Repair of Spontaneous Or Drug Induced Double Strand Breaks
Wild Type MRE11
RAD50
MRE
NBS
Repair
Mutant MRE11
RAD50
MRE
NBS
Inactive MRE11
NO Repair
DNA damage
ATM
Growth Arrest and/or Apoptosis

Figure 26



10025331 .122401

Figure 27

MRE11 Summary

Source: YTH- PCNA/Nbs1

Functional Studies

Antiproliferative Activity

- Overexpression of MRE11 H129N mutant protein is antiproliferative in tumor cells, but not in normal cells
- No strong antiproliferative effect is seen in cells expressing MRE11 wild type or H217Y mutant

Chemosensitization

- Overexpression of MRE11 H217Y mutant enhances sensitivity to chemotherapeutic treatment in tumor cells
- Sensitization by the H129N mutant cannot be assessed because of the inherant antiproliferative activity seen with expression of this mutant

Literature

- Numerous studies have suggested that MRE11 plays an important role in DNA damage repair pathway
- Studies on the yeast protein suggest that inhibition of catalytic activity of MRE11 will result in sensitivity to ionizing radiation

Conclusion

- Functional studies suggest inhibition of MRE11 will selectively inhibit tumor cell growth and enhance the response of tumor cells to DNA damaging agents

Figure 28

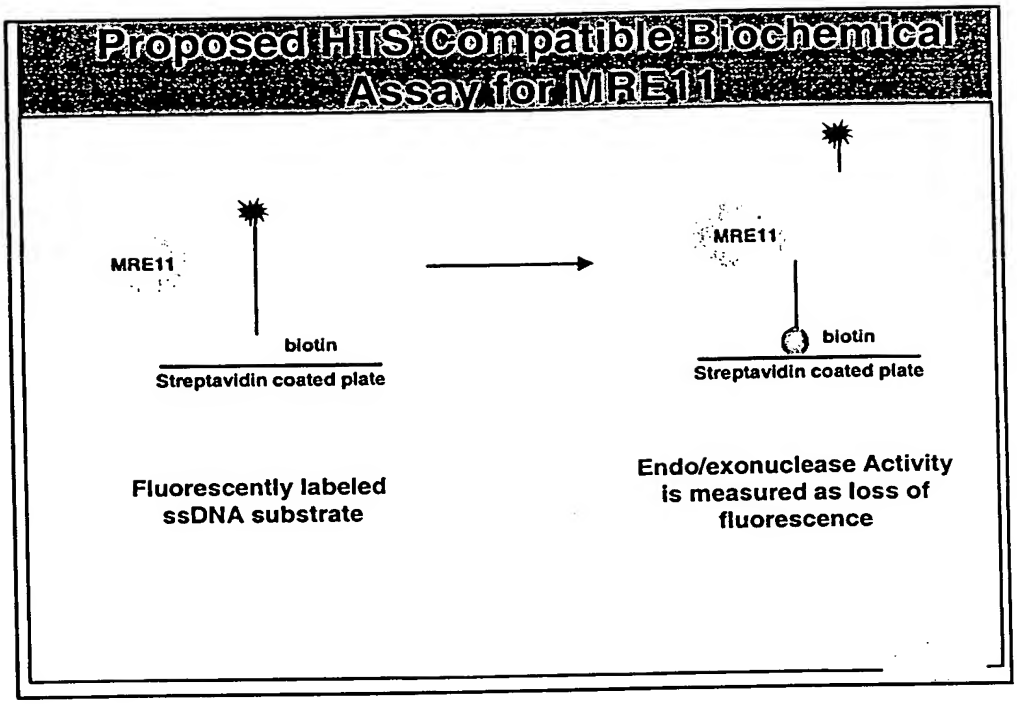
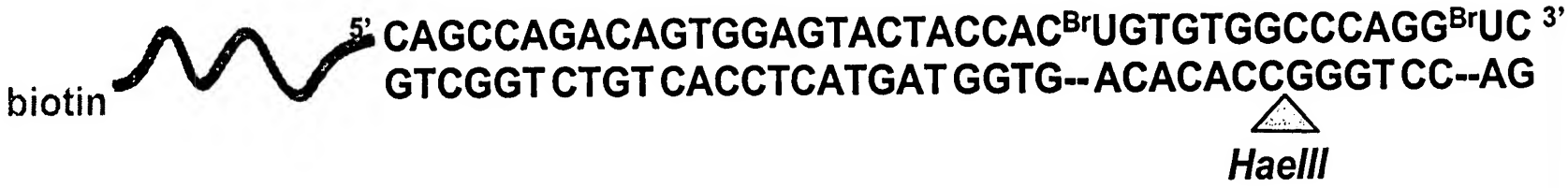


Figure 29

Oligonucleotide Duplex Substrate for Mre11 Plate-Based Assay



Sequence was taken from oligonucleotide DG51 (Paul and Gellert, Mol. Cell, 1998),a substrate used to characterize the *in vitro* nuclease activity of recombinant Mre11. A *HaeIII* cleavage site was incorporated as a positive control for the assay.

Figure 30

Biochemical Assay for Mre11 Exonuclease Activity

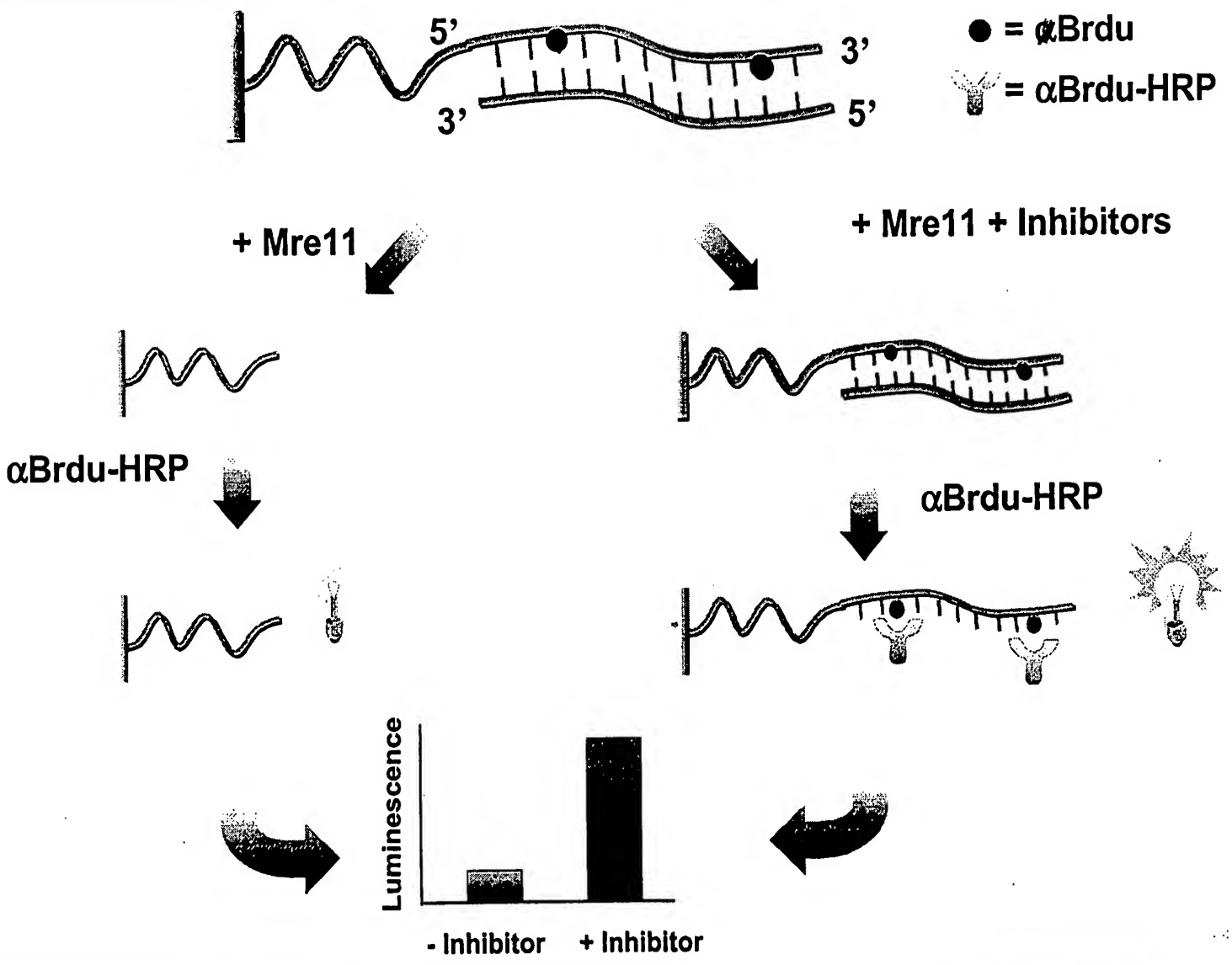


Figure 3I

Cleavage of Double-stranded Biotinylated Reporter by Mre11

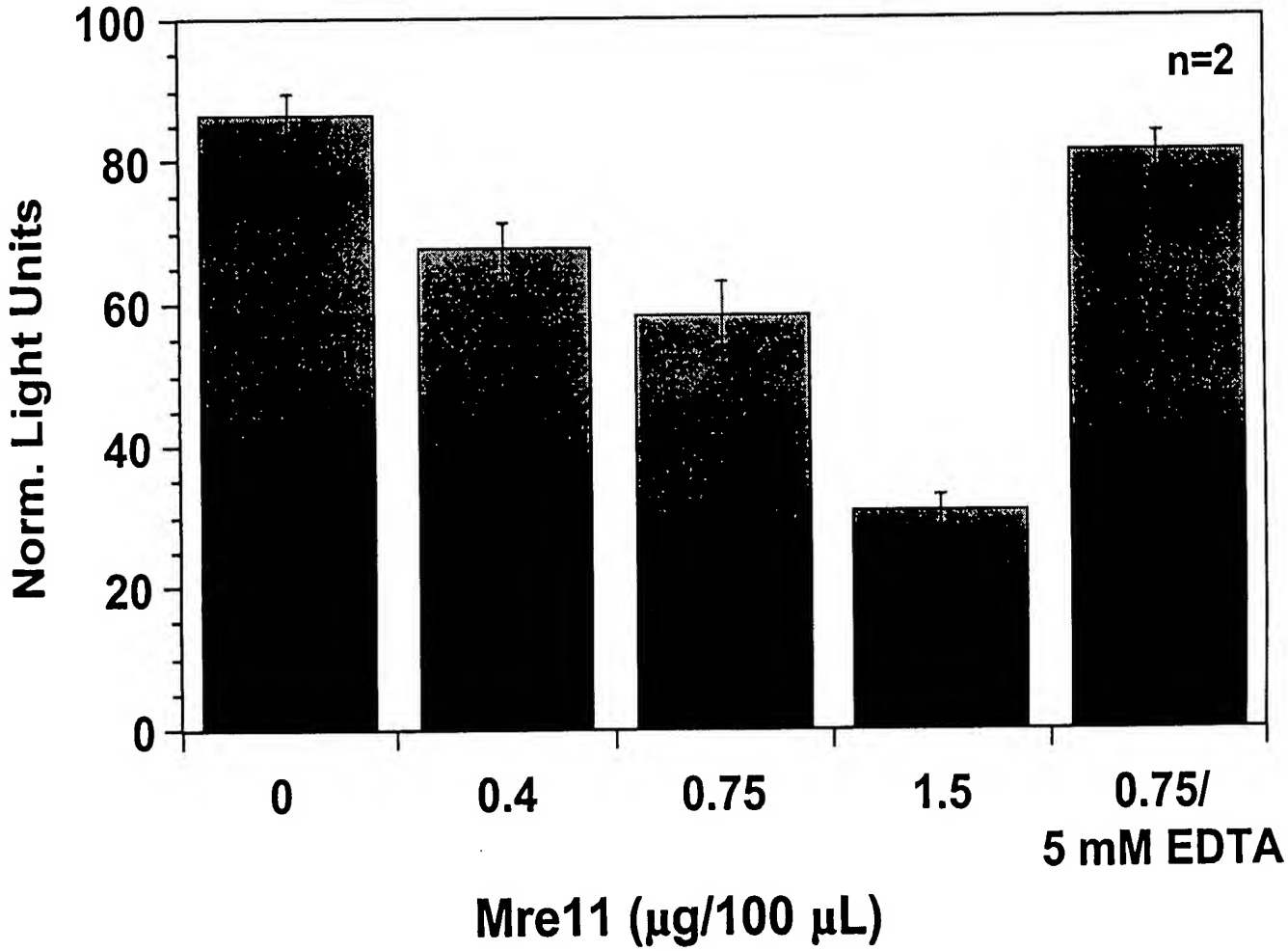
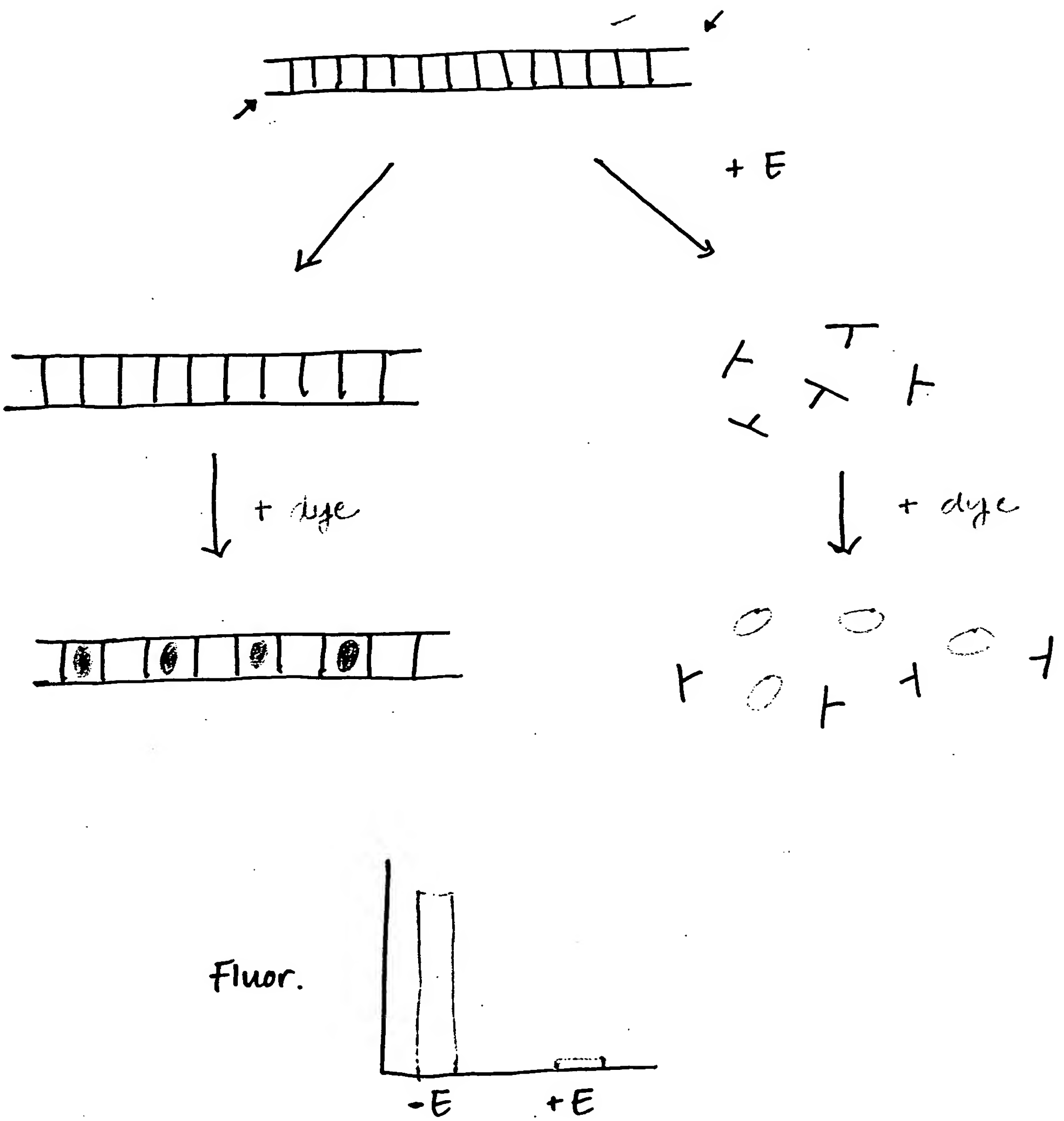
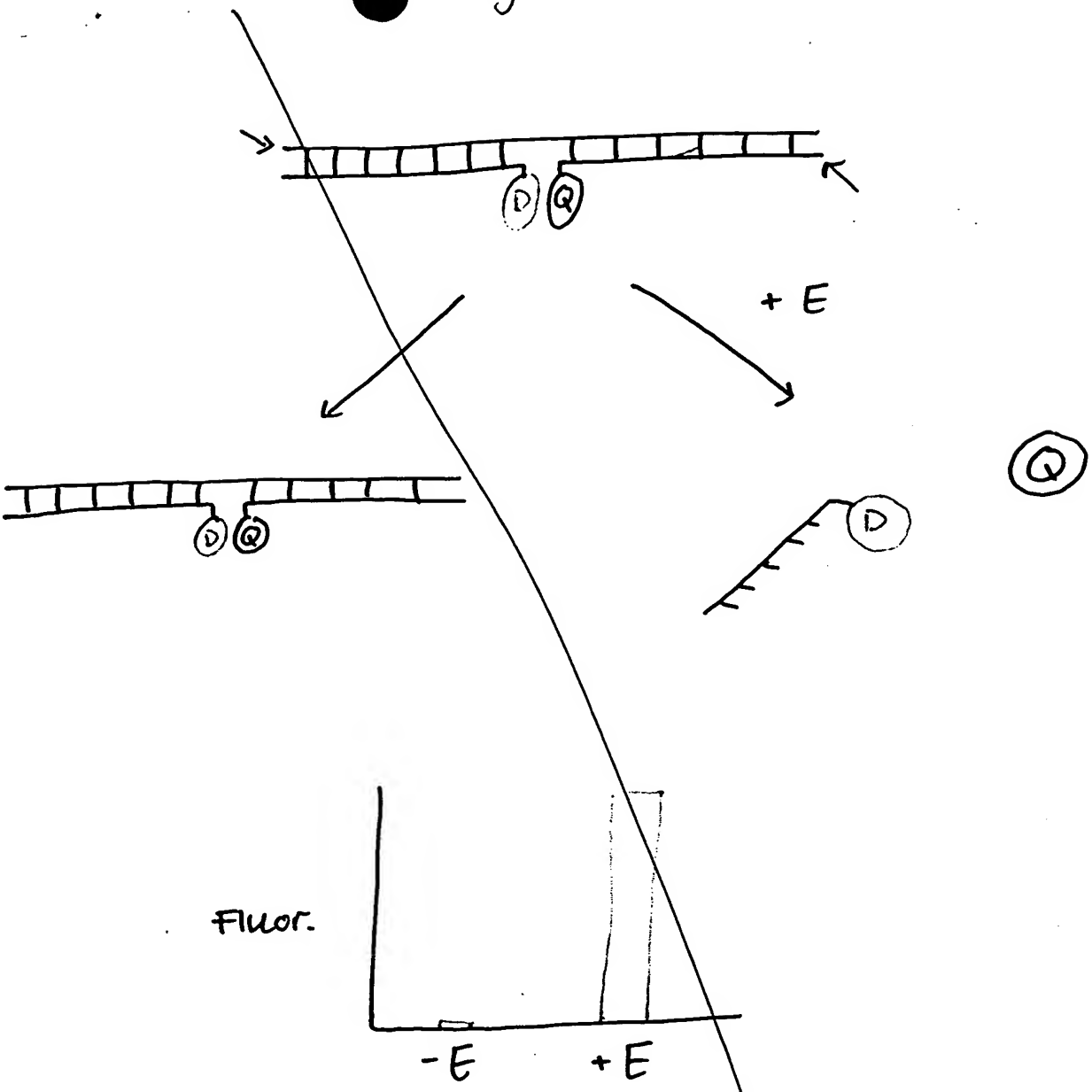


Figure 32



Picogreen Dye Assay

Figure 33



Fluorescence Quenching Assays